International Journal of Environmental Engineering and Management.
ISSN 2231-1319, Volume 4, Number 3 (2013), pp. 199-206

http://www.ripublication.com/ ijeem.htm

Mycoremediation an Eco-friendly Approach for the Degradation of Cellulosic Wastes from Paper Industry with the help of Cellulases and Hemicellulase activity to Minimize the Industrial Pollution

Amrita Singh[1] and Richa Sharma[2]

Jayoti Vidyapeeth Women`s University, Jaipur, Rajasthan.
Department of Biotechnology and Allied Science, Jayoti Vidyapeeth Women's University, Jaipur- 303007, Rajasthan.

Abstract

Environmental preservation is one of the key strategies addressing sustainable development. An awareness of environmental problems and potential hazards caused by industrial waste waters has prompted many countries to limit the discharge of toxic effluents. Mycoremediation play important role in the treatment of pulp and paper mill effluents containing numerous toxic substances like hydrocarbons, polychlorinated compounds, heavy metals, penolic-derivaties and high concentration of lignin and cellulosic materials. Fungi can easily colonize and degrade large varieties of waste e.g. waste paper, saw dust, wood chips, bagasse, black liquor of pulp and paper industrial effluents etc.. Fungi utilize some of these hazardous compounds as nutrients source and degrade or fragmenting the pollutants into non-toxic simpler forms. In the pulp and paper industry, cellulases and hemicellulases have been employed for biomechanical pulping for modification of the coarse mechanical pulp and hand sheet strength properties, de-inking of recycled fibers and for improving drainage and run ability of paper mills. Cellulases are employed in the removing of inks coating and toners from paper and also used in preparation of easily biodegradable cardboard. It is proposed to use of Hemicellulase enzymes as effective bio-reagents to achieve bio bleaching in place of poisonous chlorine compounds conventionally used to achieve pulp brightness in the manufacture of high quality paper products. The Fungal flora has been isolated from the soil of

Paper Industry. The degradation ability of fungi was checked by the fungal growth on the media containing Cellulose and Xylan as a substrate respectively. The more is the fungal growth, and more the clear zone around the colony of fungus, the more it can utilize Cellulose and Xylan and produce Cellulase and Hemicellulase enzyme. In order to remove toxic metals from soil and also to improve in treatment of industrial effluents, Mycoremediation, is emerging as potential strategy for cost-effective and eco-friendly remediation of contaminated soils.

Keywords: Pulp and Paper mill effluent; mycoremediation; cellulase; xylanase.

1. Introduction

Pulp and paper industry is considered as one of the most polluter industry in the world (Thompson *et al.*, 2001; Sumathi & Hung, 2006). The pulp and paper industry has an important activity in various countries around the world but it is considered as one of the energy intensive and highly polluting sectors and is therefore of particular concerns in the context of both local and global environmental discussions (Pokhrel and Viraraghavan, 2004). Pulp and paper production is a major industry in India, with a total capacity of over 3 million tonnes per annum (CPCB 2001). In recent times, the high cost of input energy and increased environmental concerns are forcing the pulp and paper industry to look for cost-effective and environment friendly alternatives. The nature of pulp and paper industry effluent is quite complex as it contains a number of organic components, for example, lignin, tannic acid, resin, cellulose, and hemicellulose which are difficult to be degraded (Virendra Kumar et al,2012). The great versatility of microorganisms offers a simpler, economical and more environmental friendly strategy to minimize environmental pollution and to help in biodegradation of toxic compounds. The process of using fungi for bioremediation of contaminated soils (usually) is termed as mycoremediation, coined by Paul Stamets. Mycoremediation plays a pivotal role in breaking down numerous toxic substances like petroleum hydrocarbons, polychlorinated biphenyls, heavy metals (by biosorption), phenolic derivatives, persistent pesticides etc. Fungi utilize some of these hazardious compounds as the nutrient source and convert them to simpler fragmented forms((Varsha *et al.*, 2011). Fungal treatment is lower in costs than the use of chemicals (Jalc, 2002). Over the years, a number of organisms, in particular fungi, possessing cellulose-degrading enzymes have been isolated and studied extensively (Bhat and Bhat., 1997; Nowak *et al.*, 2005; Lalitha kumara *et al.*, 2011). Biotechnological conversion of cellulosic biomass is potentially sustainable approach to develop novel bioprocesses and products. Microbial cellulases have become the focal biocatalysts due to their complex nature and wide spread industrial applications (Henrissatet *et al.,* 1998; Ramesh Chander Kuhad *et al.*, 2011).

A great variety of fungi and bacteria can fragment lignocellulosic macromolecules into simpler forms by busing enzymes. It is crucial in many applications that the enzyme mixture produced by the fungus does not contain any harmful side activities. For instance, xylanases are used for enzymatic bleaching of pulp and the enzyme preparation should not contain activities destroying the quality of the cellulose fiber (NAKARI-SETALA and PENTTILA,2005). Application of xylanase results in a lower chlorine dosage, a lower chemical cost, and lower chloro-organic concentrations in pulp and effluent (Subramaniyan and Prema ,2002;Yang et al.,1992; Bajpai et al,1994). The aim of the study to search or develop a suitable low cost biologically or environmental friendly method for bioremediation of pulp and paper mill effluent has capacity of efficient biodegradation of organic and inorganic pollutants of mill effluents.

2. Sections

2.1 Materials and Methods:

2.1.1 Study area

The study was carried out in the soil environment of Vedant Gyan Valley, Jharna village, Jaipur, Rajasthan State, India. Jharna is 48 km away from Jaipur and located on east longitude-$75^0$27’38’’ and north latitude-$26^0$49’34’’ and situated on altitude 450-500 meter above sea level.

2.1.2 Samples collections

Soil samples of 200 g were collected from the Pulp and Paper Industry. The samples were collected with small sterile shovels into sterile plastic containers. The soil samples were sent to the laboratory within 30 minutes for analysis. The pH and temperature of soil samples were determined using digital pH meter and thermometer respectively. Humidity and air pressure was determined by hygrometer and barometer respectively.

2.1.3 Isolation and identification of fungal isolates

Sabouraud’s dextrose agar (SDA) media were employed for the isolation of fungi by spread plate method using serial dilution technique. All the plates were incubated at 30°C for 7 days. Fungal isolates were identified by cultural and microscopic features.

2.1.4 Screening for Cellulase and Xylanase activities of the Isolated Fungi

The fungi were cultured on Carboxymethyl-cellulose-agar (CMC-agar) medium for Cellulase activity. This medium consist of CMC (10 g), NaNO3 (2 g), KH2PO4 (1 g), MgSO4.7H2O (0.5 g), KCI (0.5 g), FeSO4.7H2O (0.01 g), agar (20 g) and distilled water (1000 ml) and incubated at 28°C for five days and for the Xylanase activity, the composition of the medium will (g/l): birch wood xylan(1.0g), peptone (5.0g), yeast extract(5.0g),K2HPO4(0.2g) and agar (20.0g) The inoculated plates will be incubated for 5 days at 28 ± 2°C. After Incubation , the plates were stained with 1% Congo red

dye(15 min.) followed by destaining with 1 M NaCl solution for 20 min. The clear zones surrounding the colony indicate the Cellulase activity and Xylanase activity respectively.

2.1.5 Cellulase and xylanase Production

For the production opf cellulase and xylanase enzyme, the fungal cultures were inoculated grown in 100 ml of Czapek-Dox Broth medium amended with 1% cellulose and 1% birchwood xylan respectively .The pH of the medium was adjusted to 5.6. and incubated at 28^0C on a rotary shaker (100 rpm) for seven days for proper growth. The culture broth was then filtered through whatman no. 1. The fungal biomass obtained was then dried completely and weighed . the culture filterate was used as the sample for the estimation of Cellulase and Xylanase.

2.1.6 Cellulase (CMCase)assay

Cellulase (CMCase) activity was determined by mixing 1.0 ml of 1% (w/v) CMC (prepared in 50 mM Na-acetate buffer pH 5.3) with 1.0 ml of crude extracellular enzyme source and incubating at 50°C for 15 min. The reaction was stopped by the addition of 3.0 ml of 3, 5-dinitrosalicylic acid (DNS) and the contents were boiled for 15 min. The colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1 µmol of glucose equivalents per minute under the assay conditions (Mandels *et al*., 1981).

2.1.7 Xylanse assay

The amount of xylanase produced was measured by using 1% birch wood xylan as the substrate (Bailey et al., 1992). Xylanase activity was assayed in 3.0 ml of a reaction mixture containing 1.0 ml of crude extracellular enzyme source, 1 ml of 1% birch wood xylan (prepared in 0.05 M Na-citrate buffer, pH 5.3) and 1 ml of 0.05 M citrate buffer. The mixture was incubated at 55°C for 10 min. The reaction was stopped by the addition of 3.0 ml of 3, 5-dinitrosalicylic acid (DNS) and the contents were boiled for 15 min (Miller, 1959). After cooling, the color developed was read at 540 nm. The amount of reducing sugars liberated was quantified using xylose as standard. One unit of enzyme activity is defined as the amount of enzyme which releases 1 µmol of xylose in 1 min under assay conditions.

2.1.8 Estimation of total protein

Protein content of the culture supernatant determined according to the method described **Lowry *et al.*(1951**) using bovine serum albumin as standard.

Table 1: Diameter of clear zone, reducing sugar, protein and biomass by fungal isolates grown on broth

Czapek's medium (with 1% CMC) at 28°C and pH 5.6

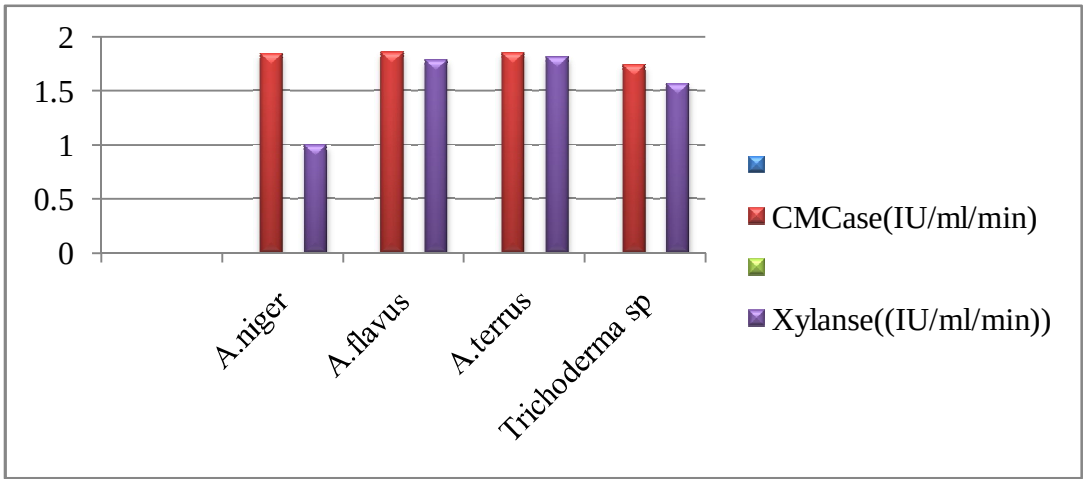
S. No.	Isoalates	Diameter of clear zones(mm)	CMCase Activity(IU/ml/min.)	Protein (mg/ml)	Biomass (mg)
1	*A. niger*	65	1.850	0.05	0.6297
2	*A. flavus*	64	1.859	0.06	0.8620
3	*A. terrus*	45	1.851	0.09	0.4499
4	*Trichoderma sp.*	70	1.743	0.01	0.6612

Table 2: Diameter of clear zone, reducing sugar, protein and biomass by fungal isolates grown on broth.

Czapek's medium (with 1% Birchwood xylan) at 28°C and pH 5.

S. No.	Isoalates	Diameter of clear zones(mm)	Xylanase activity(IU/ml/min.)	Protein (mg/ml)	Biomass (mg)
1	*A.niger*	75	0.990	0.63	0.0307
2	*A.flavus*	69	1.790	0.63	0.1200
3	*A. terrus*	57	1.818	0.84	0.1599
4	*Trichoderma sp.*	69	1.568	0.78	0.0174

Graph 1. Graph shows Comparitive study of CMCase and Xylanse activity of fungal isolates



3. Results and Discussion

The numbers of fungal species isolated from the soil samples. *Aspergillus* sp. was isolated from all the soil samples and its occurrence was also predominantly high among other fungal strains followed by *Trichoderma* sp. Isolates were screened for their ability to produce extracellular cellulases and Xylanases. The cellulase and xylanase production ability of fungi assessed by estimating zone around the colony formed due to ability of fungal isolates to hydrolyse cellulose and xylan. *Aspergillus* and *Trichoderma* were potential cellulose and hemicellulose degradors. Aspergillus flavus(1.85 IU/mg/min.;1.79 IU/mg/min) and Aspergillus terrus(1.85 IU/mg/min; 1.81 IU/mg/min) have maximum cellulase and xylanase activity as shown in Table 1 and Table 2. Biomass production of individual strain responded differently based on their different growth behaviour. *Aspergillus falvus* produces highest biomass (0.862 mg) when Czapek's Dox Broth supplemented with 1% CMC. *Aspergillus terrus* produces highest biomass (0.159 mg) when Czapek's Dox Broth supplemented with 1% xylan. Members of the fungal genus *Trichoderma* sp. and *Aspergillus* sp. have been extensively studied, particularly due to their ability to screte cellulose degrading enzyme.

4. Conclusion

An environmental problem caused by the industrial effluents is mainly due to accumulation of pollutants and other fragmented compounds. There is a quick need to degrade these pollutants in an eco-friendly way.This study revealed that lignocellulosic waste, produce large amounts of cellulase and hemicellulase enzymes when hydrolyzed by cellulolytic and xylanolytic microorganisms and instead of being left behind for natural degradation can be utilized effectively under these conditions, to produce cellulase and hemicelluase. The study also revealed that the successful colonization of fungi as decaying lignocellulosic waste in past may be correlated with their cellulolytic and xylanolytic ability. Mycoremediation was found to cover wide range of recalcitrant degradation and is known to be a better choice because of its nature of degradation.

5. Acknowledgements

The authors are grateful to department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan), for providing the laboratory facilities used in this study.

References

[1] B.Lalitha kumari, M Hanuma sri and P Sudhakar (2011). Isolation of cellulase producing fungi from soil, optimization and molecular characterization of the isolate for maximizing the enzyme yield. *World Journal of Science and Technology,*1(5) pp*. 01-09.*

[2] Bajpai B, Bhardwaj NK, Bajpai PK, Jauhari MB(1994). The impact of xylanases on bleaching of eucalyptus kraft pulp. *J Biotechnol*.,38,pp.1-6.

[3] Bhat MK and Bhat S (1997). Cellulose degrading enzymes and their potential industrial applications. *Biotechnol Adv*., 15, pp. 583–620.

[4] CPCB (2001). "Comprehensive industry document for large pulp and paper industry," COINDS/36/2000-2001, 2001.

[5] Henrissat T,Teeri T and Warren RAJ,(1998), A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants, *FEBS Letters*,425(2), pp. 352– 354.

[6] Jalc D. (2002). Straw enrichment for fodder production by fungi. The Mycota XI, Agricultural Applications. F. Kempken, Bennet, J.W. Berlin, Germany, Springer-Verlag: 19-38.

[7] Lowry, OH, Rosebrough, NJ, Farr, AL and Randal, RJ (1951), Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193,pp.267-275.

[8] Mandels M, Medeiros JE, Andreotti RE, Bissett FH. (1981) Biotechnol. Bioeng. 23, 2009.

[9] Miller, GL(1959), Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, 31,pp.426-428.

[10] Nakari-setala T and Penttila M,(2005), Production of *Trichoderma reesei* cellulases on glucose-containing media. *Applied and environmental microbiology*, 1oct. 1995, pp. 3650–3655.

[11] Nowak J, Florek, M, Kwiatek W, Lekki J, Chevallier P and Zieba E (2005). Composite structure of wood cells in petrified wood*. Mater Sci Eng*,C25, pp. 119–30.

[12] Pokhrel D, Viraraghavan, T (2004), Treatment of pulp and paper mill wastewater-a review. *Science of the Total Environment* , 333,pp. 37-58.

[13] Ramesh Chander Kuhad, Gupta R, and Singh A, (2011), Microbial Cellulases and Their Industrial Applications. *Enzyme Research* Volume 2011, pp. 10 pages

[14] Subramaniyan S, Prema P(2002), Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology and Application. *Crit Rev Biotechnol*, 22, pp.33-46.

[15] Sumathi S and Hung YT (2006), Treatment of pulp and paper mill wastes, In: *Waste treatment in the process industries.* Eds: Wang, L.K, Hung, Y.T., Lo, H.H., Yapijakis, C. pp. 453-497. Taylor&Francis. ISBN 0-8493-7233-X, USA.

[16] Thompson, G, Swain J, Kay M & Forster C (2001), The treatment of pulp and paper mill effluent: a review. *Bioresource Technology,* 77, pp. 275–286.

[17] Varsha Y.M, Naga deepti CH, and Sameera Chenna (2011), An Emphasis on xenobiotic Degradation in Environmental Clean up.*J.bioremed biodegrade* s11:001.

[18] Virendra Kumar, Purnima Dhall Rita Kumar, Yogendra Prakash Singh, and Anil Kumar(2012). Bioremediation of Agro-Based PulpMill Effluent byMicrobial Consortium Comprising Autochthonous Bacteria. *The Scientific World Journal*. pp 7

[19] Yang JL, Lou G, Eriksson KL(1992). The impact of xylanases on bleaching of kraft pulp. *Tappi J*.,75, pp.95-101.